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# RELATIONSHIPS BETWEEN BACTERIAL LEVELS AND OTHER CHARACTERISTICS OF RECREATIONAL LAKES IN THE DISTRICT OF MUSKOKA

PART 1 AEROBIC HETEROTROPHIC BACTERIA

PART 2 TOTAL COLIFORM BACTERIA

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CHARACTERISTICS OF RECREATIONAL LAKES IN THE DISTRICT OF MUSKOKA.

PART 1 - AEROBIC HETEROTROPHIC BACTERIA

PART 2 - TOTAL COLIFORM BACTERIA

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## PREFACE

Small freshwater lakes in Ontario were examined from 1970 to 1974 inclusive for the Ontario Ministry of the Environment Recreational Lakes Program. The objectives for bacteriological studies were to document the present bacteriological water quality of a number of developed recreational lakes and of one undeveloped lake. This was achieved by determining the density and distribution of fecal bacteria, and the location and sources of major bacterial inputs, in these lakes. The program provided much needed information in the field of bacteriological limnology. Reports on individual lakes were completed by 1975.

The purpose of future studies will be to determine the relationship between lakeshore development and the bacterial densities in lakes. The project to examine this relationship, known as Lakeshore Capacity, requires, at the outset, a reevaluation of the traditional bacteriological water quality parameters. This report is one of a series in which such conclusions and new approaches to future studies will be derived from the survey data.

### SUMMARY

Aerobic heterotrophic bacteria (HB) were determined in Muskoka recreational lakes waters from 1971 to 1974 inclusive. Three methods were used, a total coliform background determination, a 20°C plate count on black membrane filters, and a 20°C colony count on modified Foot and Taylor agar. The spring bacterial densities for the main body of lake water were correlated significantly ( $P = 0.01$ ) with the lake trophic status, as measured by mean summer chlorophyll (Chl a). The medium judged to be the best for this purpose was modified Foot and Taylor, the spring values for which were related to lake trophic status by the following linear regression:

$$\text{Log}_{10} \text{ HB (spring)} = 4.46 + 1.80 \text{ Log}_{10} \text{ Chl } \underline{a} \text{ (r = 0.92)}$$

This relationship was used to compare lakes; and to predict heterotrophic bacterial densities from the mean chlorophyll value, or to estimate the change in heterotrophic bacteria brought about by a change in trophic status of the lake.

Total coliform bacteria (TC) were determined in Muskoka Lakes, in the same period by a count of dark red colonies with sheen on m-Endo LES agar. Densities of TC ranged from 3 to 1,610 per 100 ml, with the largest seasonal variations in lakes of high trophic status. Maximum survey values were found mainly in the summer but occasionally occurred in the spring or fall. Maximum TC levels regressed significantly with mean summer chlorophyll (Chl a) and depth.

$$\text{Log}_{10} \left[ \frac{\text{TC (Max)}}{\text{Z (Max)}} \right] = -0.67 + 1.91 \text{ Log}_{10} \text{ Chl } \underline{a} \text{ (r = 0.93)}.$$

These data were presented as a basis for the comparison of lakes using bacteriological values. In addition, TC levels also correlated significantly with fecal streptococcus and TC Background Bacteria levels in lakes and these

relationships could also be used to compare lakes. A change in the proportions of fecal bacteria upon entering the lakes was detected by comparing correlations of bacteria at point inflows and in the main body of water. Total coliforms may be retained as an additional parameter of fecal pollution at point inflows (mainly streams) in the summer only. Maximum survey levels to total coliforms in the main body of water seemed best explained in terms of lake trophic status. About one lake in six had levels of bacteria which exceeded the Recreational Criteria and thus some kind of bacteriological problem was indicated. Inputs of total coliforms were mainly from inflowing streams. A strong seasonal variation was observed in the frequency of the other inputs which were found along the shoreline. The significant increase in frequency of pollution by total coliforms in the fall of the year was associated with the developed portion of the shoreline.

#### ACKNOWLEDGEMENTS

Many Ministry of the Environment personnel participated in the program since its inception. Members of the Biology Section provided the sampling crews and field support, while members of the Microbiology Section provided analytical crews for the mobile laboratories. The technical support of these numerous people is gratefully acknowledged. Scientific supervision was provided by Mr. E. Leggat and Mr. A. Burger. T. G. Brydges, L. T. Vlassoff, A. Burger and J. E. Pagel provided valuable comments which improved the clarity of the original manuscript.

## PART 1

### The Interpretation of Densities of Aerobic Heterotrophic Bacteria in Recreational Lakes in the District of Muskoka.

## INTRODUCTION

The interpretation of bacterial densities in water from a variety of lakes is made difficult by the lack of a basis of comparison of the values. Each new lake represents an unknown situation, and the bacterial levels appear somewhat arbitrary and unrelated to other limnological measurements.

Heterotrophic bacteria (HB) are those which require some organic carbon for their growth. Early studies, quoted by Welch (36), showed that larger numbers of heterotrophic bacteria were found in lakes of high trophic status. Anthony and Hayes (3) reported that variation in levels of aerobic heterotrophic bacteria in sediment samples from different lakes were significantly explained by the following equation:

$$\text{Log}_{10} \text{HB (Sediments)} = 0.568 + 0.513 \text{ Log}_{10} \text{ Colour} + 0.019 \text{ Log}_{10} \text{ Alkalinity.}$$

The importance of their work cannot be overemphasized, but the use of other parameters could be advantageous. Their results were important for they showed that the bacterial levels were mathematically related to other limnological parameters.

An equation relating numbers of HB to Biochemical Oxygen Demand (BOD) of polluted waters has been reported (35), and, in addition, the following relationship was found between direct counts (DC) and BOD of stagnant or running water.

$$\text{Log}_{10} \text{DC} = 5.26 + 1.17 \text{ Log}_{10} \text{BOD.}$$

It therefore appears that mathematical relationships can be found relating numbers of bacteria to various parameters of nutrient levels in water.

Many procedures have been used over the years to measure HB, and three methods were used during the Recreational Lakes Program. The first was the use of total coliform background organisms (BKGD) which are those non-coliform organisms grown on MF-Endo-LES medium (2). High BKGD levels indicated high plate count results in raw water samples examined by the MOE (9). The second method, used in 1973, was the determination of HB on a plate count medium with black membrane filters. This method had been used previously by the MOE (5). Lastly, a total count of colonies by an agar plate count method with incubation at 20°C, used for a Toronto Harbour Study (5), was modified for Recreational Lakes use in 1974. BKGD data was collected from 1971 through 1974, but none of these data were used in Recreational Lakes Program reports.

The identification of the bacteria cultured on these media from water samples cannot be fully discussed here. Samples of bacteria representing BKGD organisms (5); bacteria cultured on black membranes (20), and bacteria from Foot and Taylor agar (33) have been identified to genus levels.

The black membrane technique was discontinued when it was found that the membranes inhibited the growth of colonies of HB. This report is a comparison of the results obtained by the remaining two methods, and the introduction of a new method of interpretation of the densities of aerobic heterotrophic bacteria in Recreational Lakes.

## METHODS

### Aerobic Heterotrophic Bacteria

1. BKGD. Non-sheen colonies were enumerated on a membrane filter Millipore HAWG) cultured on m-Endo agar after the total coliform determination was completed. A geometric mean was calculated for all sampling locations in the main body of water for each five-day survey. Stations monitoring inflows and the outflows were not included. Those stations selected usually formed Group A, i.e. the main homogeneous body of water (26).
2. Agar Plate Count. A total plate count was obtained from surface inoculated plates of a modified Foot and Taylor medium, used previously by the MOE (5) and with further modifications of 20 g agar, 3.0 g peptone, 0.5 g casein per litre and 100 ppm actidione (D.M. Young and G.D. Jenkins - unpublished data - Appendix). The plates were incubated for seven days at 20°C. A geometric mean density was calculated for the mid-lake locations only.
3. Chlorophyll a. Water samples, collected for the Limnology Section, from mid-lake stations through the photic zone were stabilized with  $\text{MgCO}_3$  suspension, filtered onto a membrane filter of 1.2  $\mu\text{m}$  pore size, then stored on ice. Chlorophyll a was determined by the MOE Chemistry Section using the method of Richards and Thompson (31). The results are expressed as an arithmetic mean summer Chl a concentration.

All available data from lakes in the District of Muskoka were used for this report, and nearby additional Simcoe lakes, Bass and St. John, were added to give better representation from lakes of higher trophic status.

## RESULTS

The densities of BKGD organisms found in Muskoka Lakes are given in Table 1. In most lakes the BKGD levels increased about 40 fold from spring to summer, while in some lakes of higher trophic status, i.e. higher Chl a levels, like Maclean and St. John, this increase was often only 10 fold or less.

The total plate counts on modified Foot and Taylor agar are given in Table 2. The densities were usually higher in the spring than in summer. Variation between summer and spring data was highest in lakes of high trophic status.

A simple regression of log HB on log Chl a was calculated. The regressions of spring bacterial densities on Chl a were statistically significant and were compared by their correlation coefficients (Table 3). The equations relating heterotrophic bacterial densities to Chl a in recreational lakes are as follows:

$$\text{Log}_{10} \text{ SPRING BKGD} = 1.32 + 1.20 \text{ Log}_{10} \text{ Chl } \underline{a} \dots\dots (\text{Fig. 1})$$

$$\text{Log}_{10} \text{ SPR, SUM BKGD} = 1.12 + 0.89 \text{ Log}_{10} \text{ Chl } \underline{a} \dots\dots (\text{Fig. 2})$$

$$\text{Log}_{10} \text{ SPRING HB} = 4.46 + 1.80 \text{ Log}_{10} \text{ Chl } \underline{a} \dots\dots (\text{Fig. 3})$$

Spring data for the methods were compared (above), and it was noted that the agar plate count method gave the greatest response, judged by the largest intercept and slope, to lakes of differing trophic status.

FIGURE 1 - SPRING TC BACKGROUND VS  
TROPHIC STATUS OF SIXTEEN MUSKOKA LAKES

GEOMETRIC MEAN DENSITY OF SPRING T.C. BACKGROUND PER 100 ML

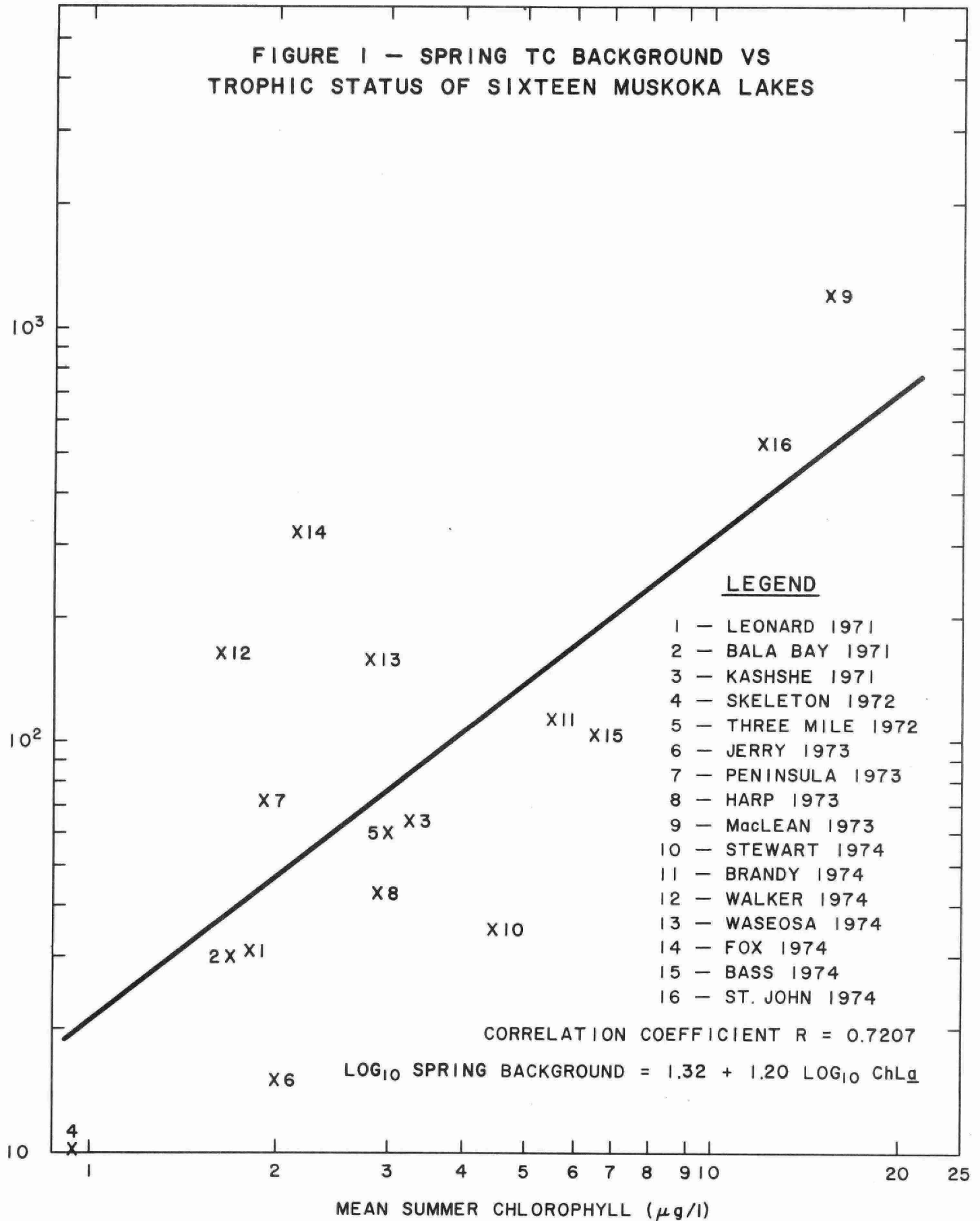




FIGURE 2 - SPRING/SUMMER MEAN TC BACKGROUND VS  
TROPHIC STATUS OF SIXTEEN MUSKOKA LAKES

GEOMETRIC MEAN DENSITY OF SPRING/SUMMER T.C. BACKGROUND PER 100 ML

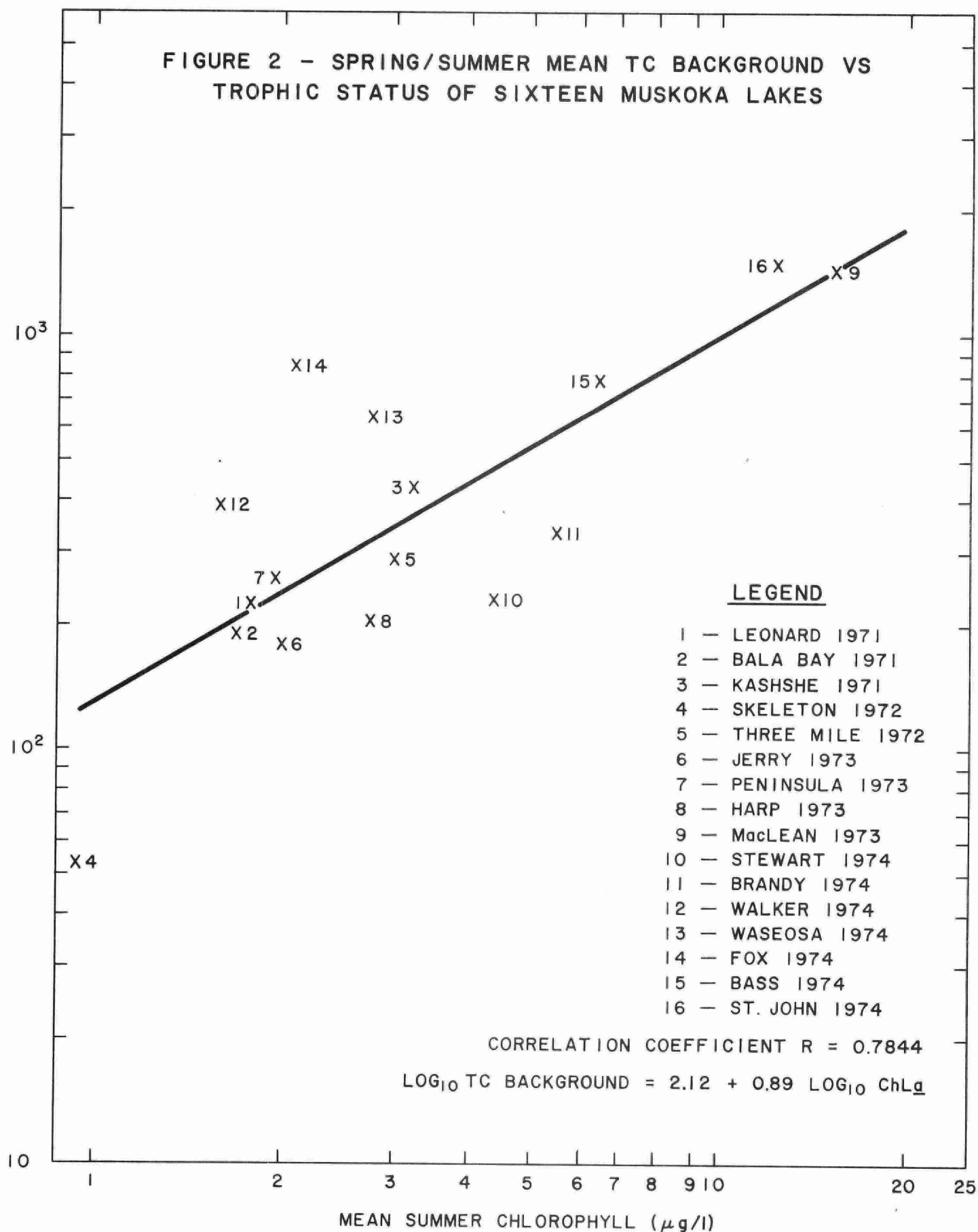


FIGURE 3 - COMPARISON OF LAKES - SPRING HETEROTROPHIC BACTERIAL DENSITY IN SURFACE WATERS VS TROPHIC STATUS

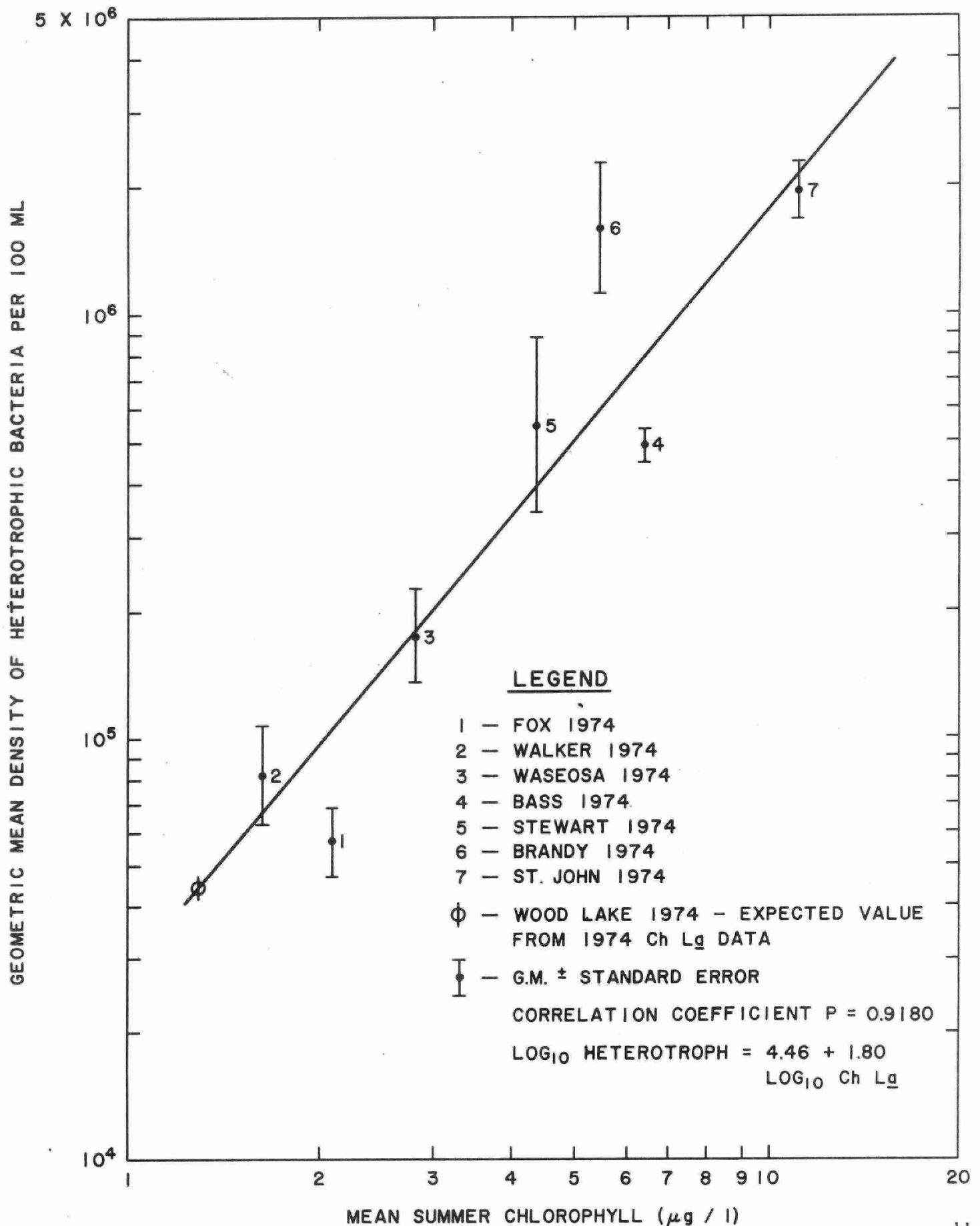


TABLE 1

TC BACKGROUND (BKGD) per 100 ml

<u>LAKE</u>	<u>SPRING GM</u>	<u>SUMMER GM</u>	<u>SPR,SUM GM**</u>	<u>Chl a ug/l*</u>
Leonard '71	32	1566	224	1.8
Bala Bay '71	30	1189	189	1.7
Kahshe '71	64	2967	436	3.3
Skeleton '72	10	279	53	0.9
Three Mile '72	60	1359	286	3.0
Jerry '73 †	15	2113	179	2.6
Peninsula '73	72	943	261	1.9
Harp '73	43	968	204	2.8
Maclean '73	1216	1766	1469	15.5
Stewart '74	35	1522	231	4.4
Brandy '74	112	1056	344	5.5
Walker '74	166	912	389	1.6
Waseosa '74	157	2597	638	2.8
Fox '74	324	2151	834	2.1
Bass '74	103	6034	787	6.4
St. John '74	517	4302	1493	12.4

\* Data obtained from Recreational Lakes Reports

† Undeveloped Lake

\*\* Geometric mean of the spring and summer values

TABLE 2

Agar Plate Count Heterotrophs per 100 ml x 10<sup>-5</sup>

<u>LAKE</u>	<u>SPRING GM</u>	<u>SUMMER GM</u>	<u>SPR,SUM GM</u>	<u>Chl a ug/l</u>
Waseosa 1974	1.74	-	-	2.8
St. John 1974	19.50	24.00	21.64	12.4
Bass 1974	4.95	0.98	2.20	6.4
Stewart 1974	5.58	0.43	1.55	4.4
Fox 1974	0.57	0.23	0.36	2.1
Brandy 1974	16.01	0.84	3.67	5.5
Walker 1974	0.82	1.02	0.91	1.6
Clearwater 1974	4.33	-	-	4.5*
Wood 1975	0.44*	0.31	-	1.3

\* Value predicted From Figure 3

TABLE 3

Correlation of Bacterial Densities with Mean Chlorophyll a

Heterotroph Method	Lakes & Stations	Correlation Coefficient	
		Spring GM	Spr, Sum GM
BKGD '71-74	16 Muskoka (all stations except inflows & outlet)	0.7202**	0.7844**
Modified Foot and Taylor '74	7 Muskoka (mid-lake stations)	0.9180**	0.9007*
BKGD	same as Foot and Taylor	0.1054 NS	-

\*\* Significant at P = 0.01

\* Significant at P = 0.05

NS Not significant at P = 0.05

## DISCUSSION

### Variation in Density of Heterotrophic Bacteria

Most agar plate count data were lower in the summer than in the spring, in agreement with previously published work (19). In contrast, all BKGD data were higher in the summer. BKGD organisms were cultivated at a higher temperature (35°C) than the larger group of heterotrophs (HB at 20°C), and it is likely that BKGD organisms were selected for by high summer lake temperatures.

Distribution of HB in surface lake water was examined in 1975 in Morrison Lake (30), and Little Panache (28), while distribution of BKGD organisms was determined in Lake Manitouwabing. In these studies the highest levels of HB were found in inflowing streams, and these bacteria were distributed more uniformly in the lake than the parameters of pollution (fecal coliforms, etc.).

Spring densities of heterotrophs by the agar plate method ranged from  $0.57 \times 10^5$  to  $19.5 \times 10^5$  per 100 ml. It was noted that the levels of heterotrophs in all mesotrophic and eutrophic lakes studied were at times greater than the permissible raw drinking water standards (16) of  $1.0 \times 10^5$  per 100 ml (Table 8). It follows that the water from the more enriched Muskoka lakes will require more treatment than usual to produce a safe drinking water.

The total number of bacteria present in water can be measured by a direct microscopic count, and these bacteria can range approximately between  $10 \times$  to  $1000 \times$  as great as the total heterotroph density. The total bacteria in Muskoka lakes must approach a density great enough to contribute to the turbidity of the water. Many bacteria in water adhere to particles, algal cells and clay, which themselves make the water turbid, and it is difficult to calculate the contribution of the bacterial cells. It would be interesting to evaluate the contribution to water turbidity of the bacteria present in lake water, but a suitable method does not appear to be available at present.

Relationship of Heterotrophic Bacteria to Lake  
Trophic Status

Heterotrophic bacteria in freshwater lakes usually show a summer minimum and spring and fall maxima, which are associated with pulses of plankton production (19). In eutrophic lakes, a summer plankton peak is likely as well. These observations of Henrici offer an explanation as to why our spring data was more meaningful, and was significantly correlated with mean Chl a. Algae are known to leak organic substances on which bacteria grow (1), and bacteria excrete compounds which stimulate algal growth (13). Factors, like temperature or sunlight, which affect the excretion of these products, will influence this type of relationship. The bacteria and algae nourish each other to some extent, although the precise interrelationship cannot be described at present (21).

This work on recreational lakes appears to be the first to report a mathematical relationship between HB found in water of small lakes, and the lakes' trophic status measured by mean summer Chl a. This relationship was:

$$\text{Log}_{10} \text{ Spring HB} = 4.46 + 1.80 \text{ Log}_{10} \text{ Chl } \underline{a}.$$

More than one explanation for the observed relationship between HB and Chl a in lakes can be found. The precise explanation need not be considered to be the most important point, rather it should be thought that if the relationship is reproducible then it may be useful.

The organic content of lake water rises as the colour increases, and so one would expect higher levels of heterotrophic bacteria in coloured lakes. The effect of this can be seen in our data. For example, Brandy Lake was the most highly coloured lake in the series studied by the agar plate count methods, and appeared to have higher levels of HB than its Chl a level would predict. Therefore, a better relationship would likely be found by using the following multiple regression:

$$\text{Log}_{10} \text{ Spring HB} = A + B \text{ Log}_{10} \text{ Chl } \underline{a} + C \text{ Log}_{10} \text{ colour}$$

(where A, B and C are constants).

Colour measurements were not available for the lakes under study.

The Chl a term in the above equation is of great importance for it sums up the effects of many environmental factors which influence a lake. Presumably one can relate bacterial densities to these environmental factors by suitable substitutions for the Chl a term.

BKGD organisms interfere with total coliform determinations in southern Ontario Lake waters (see Part 2). The probability of interference will be greatest in lakes of highest trophic status where the BKGD levels is highest (Fig. 2).

#### FUTURE USES OF THE HETEROTROPHIC BACTERIA/TROPHIC STATUS RELATIONSHIP

##### 1) Comparison of Lakes

A method of comparing lakes was developed by the Biology Section of the MOE and was used in most recreational lakes reports, where it was presented as a chlorophyll, secchi disc curve. This work shows that the bacterial values can be compared on a similar curve of paired HB and Chl a values. This approach has been used in a recent environmental impact statement (29), and was presented in a similar manner to the data in Figure 3.

The chlorophyll, secchi disc relationship, though it was not used in this form, could be written as follows:

$$\text{Log}_{10} \text{ SD} = a - b \text{ Log}_{10} \text{ Chl } \underline{a} \text{ (where SD is the secchi-disc depth).}$$



If lake water is coloured, then lower secchi disc values are obtained than would be predicted from the Chl a value alone, and a better relationship could probably be obtained from the following relationship:

$$\text{Log}_{10} \text{SD} = a + b \text{Log}_{10} \text{Chl } \underline{a} + c \text{Log}_{10} \text{Colour}.$$

This exercise is merely to show the similarity in the form of the relationship between HB, Chl a and SD, Chl a.

2) Prediction of Mean Summer Chlorophyll from Spring Measurements of Heterotrophic Bacteria

Occasionally Chl a measurements are unreliable or are not available for a particular lake. When spring HB levels are known, an estimate of mean summer Chl a can be made from Figure 3. This is not to suggest that HB values should replace the measurements of Chl a. Predictions should be made only with lakes similar to those used in this study until more varied lakes are added. This prediction was tested with Clearwater Lake (Table 2), and the results were acceptable (Personal communication with Mr. R. Shaw).

3) Prediction of Heterotrophic Bacteria from Mean Summer Chlorophyll Values

The trophic status self-help program has generated much Chl a data without corresponding bacteriological data (32). Estimates of spring HB density could now be made from published Chl a values.

4) Lakeshore Capacity

At present, there are no models which predict the effects of cottage development, or other types of shoreline development, on the bacterial levels in a lake. Bacterial levels in lakewater are expected to increase after shoreline development, and this was deduced from comparisons with an undeveloped lake (27) and from comparisons made within developed lakes.

A model has been developed whereby the effect of cottage development on phosphorus levels, and subsequently on Chl a levels in a lake can be predicted (10). This model can be augmented so that average changes in bacterial levels following development can be predicted.

$$\text{Log}_{10} \text{HB}_2 = \text{Log}_{10} \text{HB}_1 + 1.8 \text{ Log}_{10} \Delta \text{ Chl } \underline{a}.$$

Where :

$\text{HB}_1$  = Present spring heterotrophic bacteria levels .

$\text{HB}_2$  = Predicted spring heterotrophic bacteria levels following cottage development .

$\Delta \text{ Chl } \underline{a}$  = the difference between two yearly mean Chl a values; the first taken before development, and the second taken after development.

This would give an estimated change on the average but not a prediction for a specific lake. Specificity is acquired from Dillon and Rigler's Model (10). Errors found in Dillon and Rigler's model would be added to the errors in the HB, Chl a relationship and could become unacceptably high. The method may be of use at present since no other method is yet available.

## PART 2

### The Interpretation of Total coliform Levels in Recreational Lakes in the District of Muskoka

#### INTRODUCTION

The total coliform group comprises all of the aerobic and facultatively anaerobic, gram-negative, nonsporeforming, rod shaped bacteria which ferment lactose with gas formation within 48 hours at 35°C. The method of determining total coliforms in the recreational lakes under study was a total count of dark red colonies with sheen on m-Endo LES agar (2). A few other bacteria produce sheen colonies on this medium (4, 6). As the coliforms were not confirmed by additional tests, such bacteria were included in the total coliform estimations, but were not expected to contribute large errors. The bacteria which grow on m-Endo's medium, the formulation of which is very close to m-Endo LES, were also identified in a previous MOE study (5). The type of bacteria cultured from lake samples on m-Endo LES medium were identified in a recent study of coliforms from recreational lakes (18).

In bodies of water total coliforms are used as indicators of waste pollution (2), but coliforms may come from unpolluted soil and water (8), and so their numbers may not be reliably related to the degree of pollution. An important problem, that of describing the relationships between different coliform types and their natural habitats or pollution sources, is unsolved. Coliforms are known to grow in nutrient rich waters, and for this and other reasons, they are considered by some to be an inadequate index of water quality (12).

During the Recreational Lakes Program bacteriological water quality was measured by three parameters, total coliforms, fecal coliform and fecal streptococcus, which were interpreted together to avoid the possible errors mentioned above. It appeared from this experience that, of the parameters, fecal coliforms gave the most reliable index of lake water quality. Research interest has now focused on this bacterial type and method refinements are under consideration, for example, m-TEC medium (11).

Total coliforms are heterotrophic bacteria which are distinguished by their use as indicators of water pollution. The mathematical relationships which have been proposed to explain heterotrophic bacterial levels in Muskoka Lake water (this Report - Part 1) have not been developed for total coliform bacteria. This report gives an overview of total coliform levels in Muskoka Lakes and describes the relationship of total coliforms with some other water quality parameters. If past data is to be examined, then total coliforms appear to be a useful index of water quality in Recreational Lakes, and a new interpretation is presented here. However, better parameters are suggested for future studies. Total coliforms will then have only a marginal usefulness.

## METHODS

### Selection of Lakes:

All available data from lakes in the District of Muskoka were used for this report, and nearby additional Simcoe County lakes, Bass and St. John, were added to give better representation from lakes of higher trophic status.

### Surveys:

Lakes were surveyed for five consecutive days, in the spring, summer and often the fall of the year, at a number of locations considered to be representative of shoreline features and development, In addition, one or two

sampling locations were placed in the middle of the lake. Samples were taken one metre from the surface 10-15 meters from shore, and an additional sample at a mid-lake location was taken one metre from the bottom.

#### Bacterial Enumeration:

- 1) Total coliform bacteria were determined as a count of dark red colonies with sheen grown on a membrane filter (Millipore, HAWG) with m-Endo LES agar (2).
- 2) Fecal coliform bacteria were determined as a count of acid producing yellow to yellowish-green colonies grown on a membrane filter with McConkey broth at 44.5°C.
- 3) Fecal streptococcus bacteria were obtained from a count of pink or red colonies grown on a membrane filter with m-enterococcus agar (2).
- 4) Heterotrophic bacteria were obtained from a total count from surface inoculated plates of a modified Foot and Taylor medium (Appendix).

#### Data Processing:

These data were evaluated by statistical techniques in the following manner. The geometric mean, and standard deviation were calculated for the values of each of the three bacterial types at every station, providing concise valid data. Statistically significant variations in the bacterial densities between stations, or groups of stations, were determined by a One-Way Analysis of Variance and Barlett's Test of Homogeneity. By these means the data from each station were tested against those of every other station until all stations with similar geometric mean densities were separated into groups (A, B, etc.). Sampling locations in the main body of water usually formed a large group (Group A), whereas sampling locations at the mouth of streams were rejected from this group and were treated separately. Those values which were consistently higher than the

group values for the main body of water could often be identified with sources of bacteria into the lake. Where GM values are quoted they represent the GM of five consecutive days of data. The Group A geometric mean summarized from 60 to 200 species of data depending on the size of the lake.

Chlorophyll a:

See this Report - Part 1.

RESULTS

Total coliform densities in the study lakes ranged from 3 to 1,610 per 100 ml, with the largest seasonal variations found in lakes of high trophic status (Table 4). Maximum survey total coliform densities were found mainly (11 of 17 lakes) in the summer, but a smaller number (6 of 17 lakes) were found in spring or fall. The Recreational Criteria (16) were exceeded by maximum values in three eutrophic lakes, Maclean Lake, Stewart Lake and Muskoka Bay.

The total coliform levels in the undeveloped lake, Jerry Lake, were low, but the lowest levels recorded, 3 TC per 100 ml, were found in Skeleton Lake in the spring survey. Skeleton Lake was the deepest lake in this group (Table 4).

Spring and summer total coliform levels were not correlated well enough to predict one season's survey values from the other (results not illustrated).

Correlation coefficients were calculated for pairs of water quality parameters for the main body of the lake and point inflows to the lakes (Table 5).  $\text{Log}_{10}$  transformed data gave more significant results than the original data. Total coliform levels at point inflows to the lake were highly correlated with the other bacterial parameters except spring fecal coliforms. In contrast, in the main body of the lake, total coliform densities correlated only with fecal streptococcus levels. It was also observed that correlations of bacterial values within individual lakes were greater and more often statistically significant than the correlations across the group of lakes (Table 6).

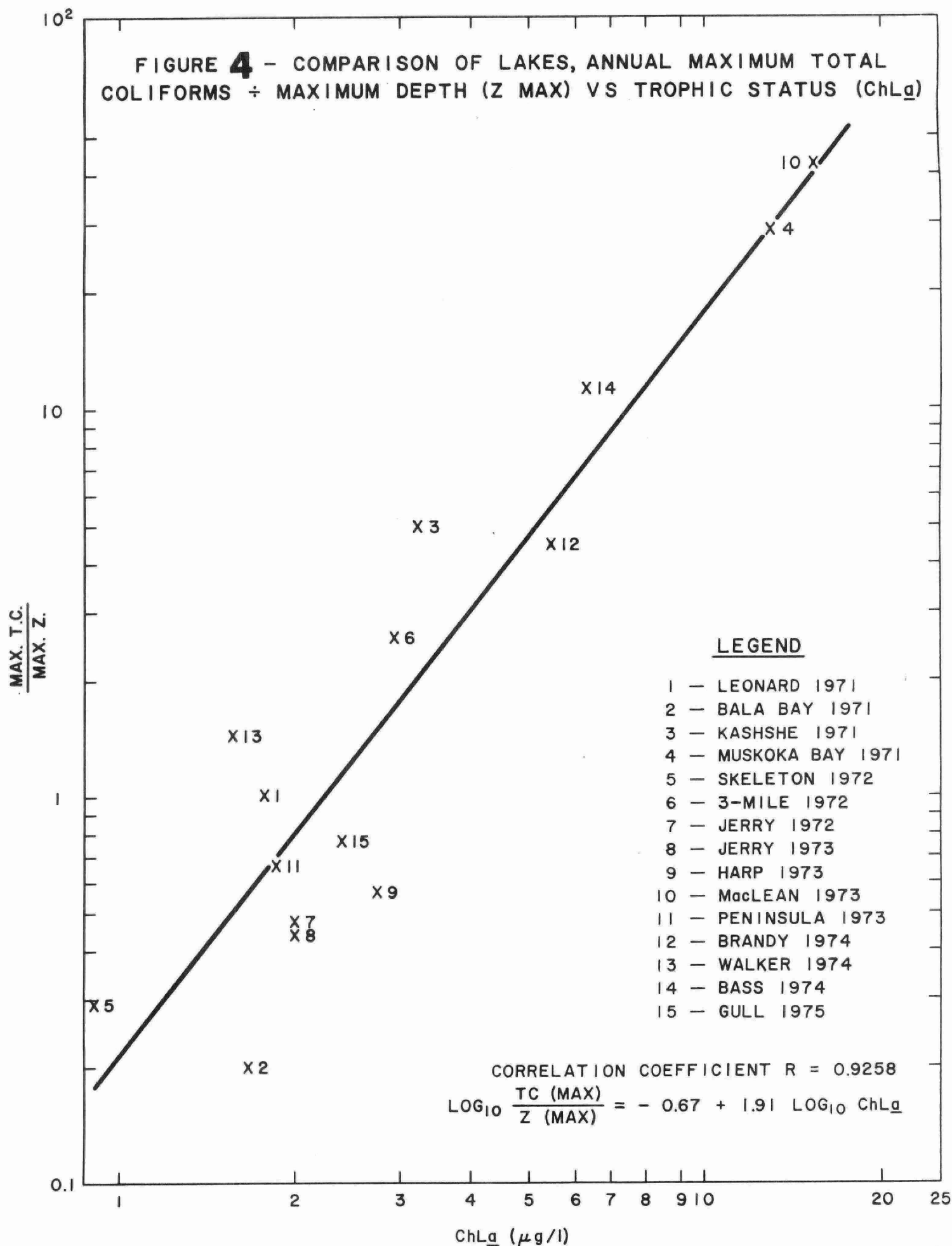
Total coliform densities for the main body of the lakes were significantly correlated with Chl a and depth (Z) (Table 7). The relationship between total coliform levels and trophic status of lakes was described by the following regression equations:

$$\text{Log}_{10} \left[ \frac{\text{TC max}}{\bar{Z} (\text{max})} \right] = - 0.67 + 1.91 \text{ Log}_{10} \text{Chl } \underline{a} - \text{Fig. 4}$$

$$\text{Log}_{10} \left[ \frac{\text{TC (Spr, Sum GM)} \times 10}{\bar{Z}} \right] = 0.56 + 1.52 \text{ Log}_{10} \text{Chl } \underline{a}$$

Further equations could be developed from the data in Table 7. The values of inputs of total coliform bacteria at stream mouths and along the shore were given in Table 11.

FIGURE 4 - COMPARISON OF LAKES, ANNUAL MAXIMUM TOTAL COLIFORMS ÷ MAXIMUM DEPTH (Z MAX) VS TROPHIC STATUS (ChL<sub>a</sub>)





TABE 4

GEOMETRIC MEAN (GM) TOTAL COLIFORM DENSITIES (TC/100 ML) FOR THE MAIN BODY OF LAKES

NO.	LAKE	SPRING	SUMMER	FALL	SPRING/SUMMER	MAXIMUM SURVEY	DEPTH		Chl <u>a</u> (ug/l)
		GM	GM	GM	GM	GM	Z max (ft.)	$\bar{Z}$ (ft.)	
1	Leonard '71	13	14	51	13.49	51	50.0	23.6	1.8
2	Bala Bay '71	10	20	24	14.13	24	122.0	?	1.7
3	Kahshe '71	38	11	326	20.45	326	66.0	28.3	3.3
4	Muskoka Bay '71	-	1600	-	-	1600	56.6	23.0	13.2
5	Skeleton '72	3	63	-	13.75	63	216.0	99.9	0.9
6	3-Mile '72	8	37	-	17.2	37	14.4	11.3	3.0
7	Jerry '72 ‡	46	56	51	40.32	56	116.6	46.0	2.0
8	Jerry '73 ‡	8	51	11	20.19	51	116.6	46.0	2.0

‡ = Undeveloped Lake

Table 4 - continued

NO.LAKE		SPRING	SUMMER	FALL	SPRING/SUMMER	MAXIMUM SURVEY	DEPTH		Chl <u>a</u>
		GM	GM	GM	GM	GM	Z max (ft.)	$\bar{Z}$ (ft.)	(ug/l)
9	Harp '73	49	68	25	57.72	68	199.9	47.6	2.8
10	Maclean '73	1520	41	23	249.7	1520	35.0	17.3	15.5
11	Peninsula '73	47	75	-	59.37	75	112.0	29.3	1.9
12	Stewart '74	51	1610	-	286.5	1610	69.0	20.6	4.4
13	Brandy '74	46	102	-	68.5	102	23.0	11.7	5.5
14	Walker '74	72	77	-	74.46	77	50.0	17.3	1.6
15	Waseosa '74	35	8	-	16.73	35	63.0	23.0	2.8
16	Fox '74	45	9	-	20.12	45	49.0	17.6	2.1
17	Bass '74	58	335	-	139.4	335	45.0	12.0	6.4
18	St. John '74	105	396	-	203.9	396	25.0	15.5	12.4

TABLE 5

Correlation coefficients for Log<sub>10</sub> of pair of bacterial water  
quality parameters for the main body of lakes and point inflows

Correlation between Bacterial parameters	<u>Source of data</u>	
	Main body of the lake	Many point inflows to the lake
Spring values		
TC vs FC	0.0186 NS	0.1605 NS
TC vs FS	0.4752 NS	0.4859 **
Summer values		
TC vs FC	0.3833 NS	0.6264 **
TC vs FS	0.7091**	0.5537 **

\*\* Significant at P = 0.01

NS - Coefficient not significant at P = 0.05

TABLE 6

CORRELATION COEFFICIENTS FOR PAIRS OF LOG<sub>10</sub> BACTERIAL WATER QUALITY  
PARAMETERS FOR SAMPLING STATIONS WITHIN LAKES

	STEWART LAKE	THREE MILE LAKE
SPRING		
TC vs. FC	- 0.0045 NS	0.3338 NS
TC vs. FS	0.3364 NS	0.4395 *
SUMMER		
TC vs. FC	- 0.0319 NS	0.4938 **
TC vs. FS	- 0.5849 *	0.5166 **

\* Significant, P = 0.05

\*\* Significant, P = 0.01

NS Not significant, P > 0.05

TABLE 7

Correlation Coefficients of Log Geometric Mean (GM) Total Coliform

Density vs. Log Chl a

BACTERIOLOGICAL SURVEY VALUE	CORRELATION COEFFICIENT
Spring GM	0.7485**
Summer GM	0.5449*
Spring, Summer GM	0.7533**
Maximum Survey GM	0.7953**
<u>GM Spring, Summer x 10</u> - Z	0.7636**
<u>Max GM</u> - Z	0.8658**

\*\* Significant, P = 0.01

\* Significant, P = 0.05

## DISCUSSION

### Variation in Total Coliform Levels

Large variations in total coliform levels were found in Muskoka Lakes and our present aim was to find a basis for these variations and to re-examine the interpretation of water quality based on total coliforms in recreational lakes.

Seasonal variations of total coliform densities in freshwater lakes show a prominent maximum in the period August to September. This was illustrated by a study in Lake Michigan (7) and Lake Ontario (5) as well as Harp Lake (26) and Jerry Lake (27) in Muskoka. Data for a whole year were, however, not available from the Recreational Lakes Program. From this previous work a total coliform maximum was expected in the summer or early fall, and was found in 14 of 17 lakes studied (Table 4). Two of the three remaining lakes which showed a spring maximum (Maclean, Fox) were shallow with rapid flushing rates.

### Effect of Temperature and Nutrients on Total Coliform Levels

The growth of total coliforms in water is regulated by nutrient levels and temperature (24). Lake water temperature reaches a maximum in late August. The observed summer maxima of total coliforms are likely caused by high water temperatures. Without nutrients, however, elevated temperatures lead to more rapid death of the bacteria, for starved coliforms survive longer at low temperatures (15). High summer total coliform densities in lakes are therefore likely related to the nutrient content and temperature of lake water.

### Relationship of Total Coliform to Lake Trophic Status

In a reservoir in England (34) total coliforms increased rapidly after an algal bloom, but it was not known if nutrients for growth were supplied by the algae or merely accompanied them. In Muskoka Lakes total coliforms correlated significantly with mean summer Chl a (Table 7). The most likely explanation is that conditions for growth of coliforms were present in Muskoka Lakes, so that

nutrients available to coliforms entered the lakes in reasonable proportion to the phosphorus which nourished the algae. Leakage products from algae or degraded algae may nourish certain types of total coliforms. At present, it is not possible to decide if the relationship between total coliforms and algae (Chl a) is direct or indirect. A similar relationship between summer Chl a levels and total coliform densities was noted in lakes studied for the recreational lakes self-help program (17). The phosphorus input to Muskoka Bay was reduced in recent years with a corresponding reduction in algae (23). It would be interesting to resurvey Muskoka Bay for total coliforms and determine if new values are in proportion to the lowered Chl a levels.

In summary, in Muskoka Lakes the maximum total coliform levels are found largely in August and September, and are therefore correlated with maximum lake water temperature. They are also highly related to lake trophic status, as described by the following regression equation:

$$\text{Log}_{10} \left[ \frac{\text{TC (max)}}{\text{Z (max)}} \right] = -0.67 + 1.91 \text{ Log}_{10} \text{ Chl } \underline{a} \text{ (Fig. 4).}$$

Rearrangement showed this to be a multiple regression of the form:

$$\text{Log}_{10} \text{ TC (max)} = -0.67 + 1.91 \text{ Log}_{10} \text{ Chl } \underline{a} + \text{Log}_{10} \text{ Z (max).}$$

It was difficult to explain why total coliform densities were related to the lake depth. The above equation states that as lake depth increases, the total coliform levels are greater than would be predicted from the Chl a levels alone. Lake temperature is related to depth with deep lakes being colder lakes. It was noted earlier that coliforms survived better at cold temperatures so that coliform levels and depth could be related through lake temperature.

A word of caution is required. Lake depth may have functioned in the above equation because it was indirectly related to the real causative factor which may be flushing rate which is generally smaller in deeper lakes. A final conclusion awaits a more complex analysis

Maximum depth was also related to trophic status in these lakes.

$$\text{Log}_{10} Z (\text{max}) = 2.04 - 0.51 \text{ Log}_{10} \text{ Chl } \underline{a}.$$

#### Relationship Between Total Coliforms and Other Water Quality Parameters

Correlations between total coliforms and other bacteriological water quality parameters are reported infrequently, but a few examples can be given (7), (14), (17). In Muskoka Lakes total coliforms correlated significantly only with fecal streptococcus and then only in the summer. An improvement was found in this correlation for the main body of water over point inflows (Table 5). Accurate predictions could not be made from this relationship, though a rough estimate may be possible.

$$\text{Log}_{10} \text{ FS (Summer)} = 0.03 + 0.40 \text{ Log}_{10} \text{ TC (Summer)} - \text{Fig. 5.}$$

Heterotrophic bacteria did not correlate with TC values. A correlation was expected as both parameters correlated with chlorophyll. There were only HB data from seven lakes so that a high correlation coefficient was required before significance was obtained. This was likely the main reason for the lack of significance between HB and TC values.

In support of this, it was shown that spring BKGD values, which were available for all lakes, correlated with spring TC densities for the main part of the lakes. This group of bacteria (BKGD) was used previously as a measure of heterotrophic bacteria (this report - Part 1). The BKGD, TC relationship was poor in the summer when BKGD levels rose by about 40 fold, however, in the spring the relationship was good: ( $R = 0.8591$ ) (Fig. 6).



FIGURE 5 - RELATIONSHIP BETWEEN LEVELS OF SUMMER FECAL STREPTOCOCCUS VS SUMMER TOTAL COLIFORM IN 16 MUSKOKA LAKES

LEGEND

- 1 - LEONARD 1971
- 2 - BALA BAY 1971
- 3 - KAH SHE 1971
- 4 - SKELETON 1972
- 5 - THREE MILE 1972
- 6 - JERRY 1973
- 7 - HARP 1973
- 8 - MACLEAN 1973
- 9 - STEWART 1974
- 10 - BRANDY 1974
- 11 - WALKER 1974
- 12 - WASEOSA 1974
- 13 - FOX 1974
- 14 - BASS 1974
- 15 - ST. JOHN 1974
- 16 - PENINSULA 1973

- 33 -

SUMMER FS/100 ML

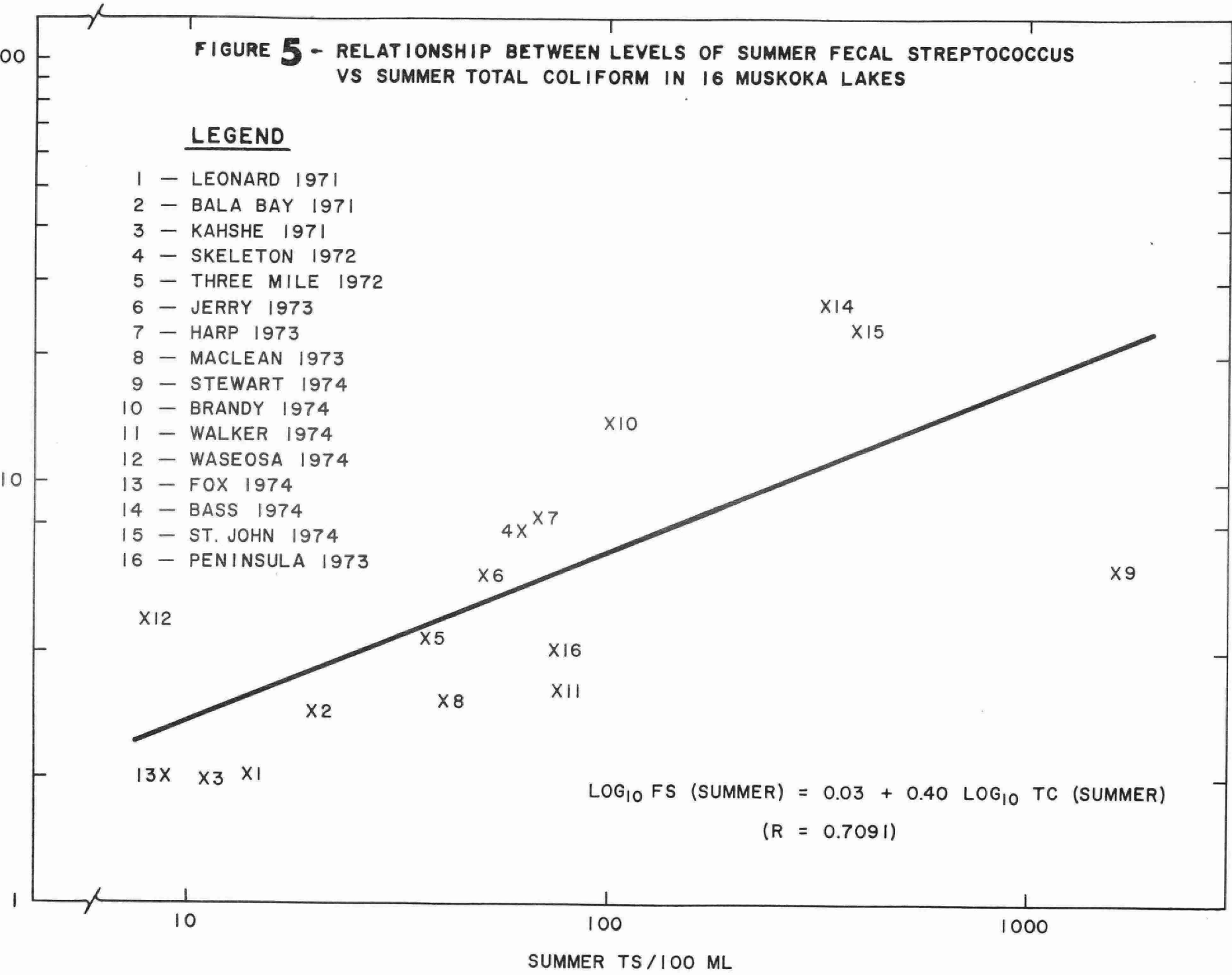
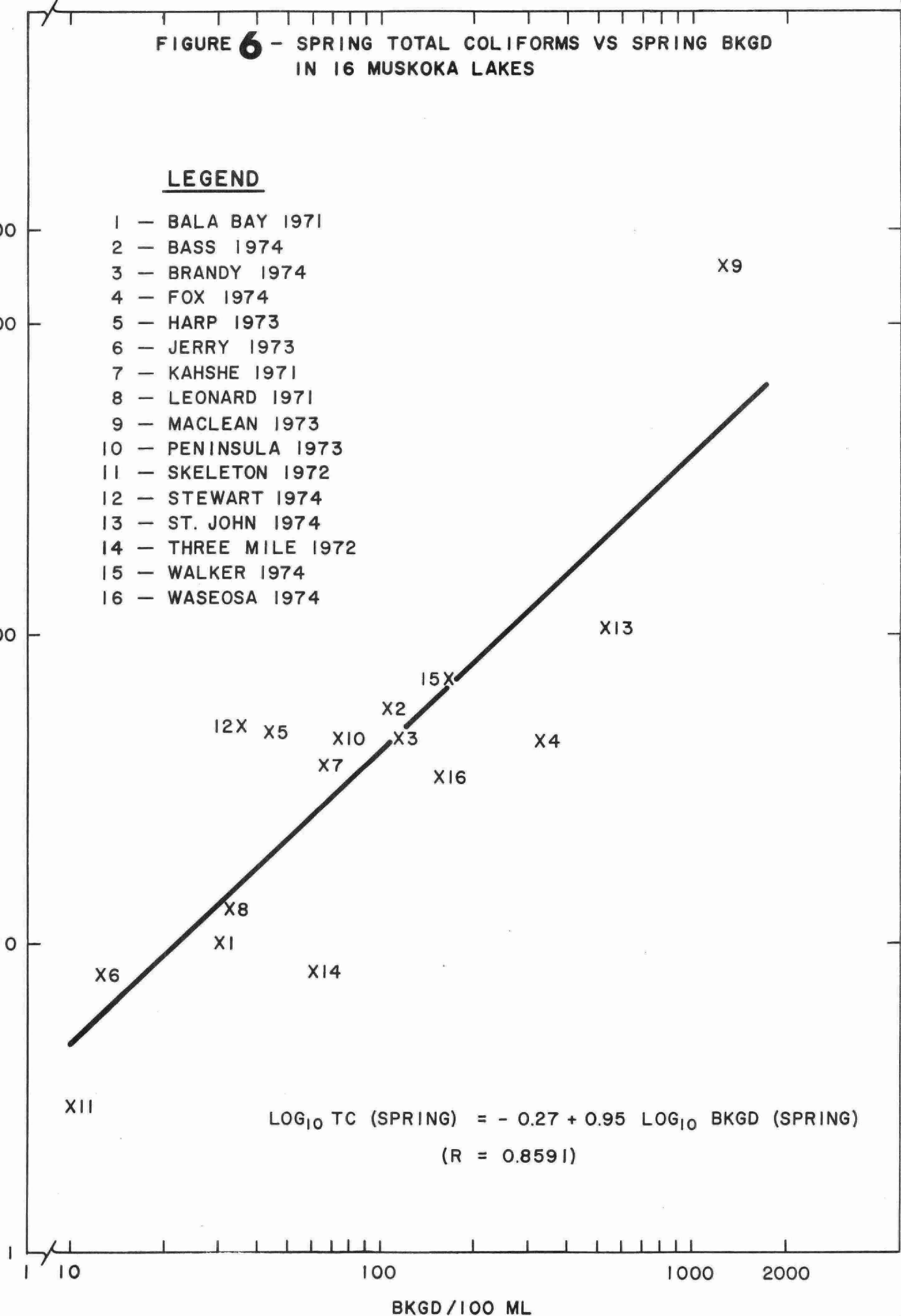


FIGURE 6 - SPRING TOTAL COLIFORMS VS SPRING BKGD  
IN 16 MUSKOKA LAKES

LEGEND

- 1 - BALA BAY 1971
- 2 - BASS 1974
- 3 - BRANDY 1974
- 4 - FOX 1974
- 5 - HARP 1973
- 6 - JERRY 1973
- 7 - KAHSE 1971
- 8 - LEONARD 1971
- 9 - MACLEAN 1973
- 10 - PENINSULA 1973
- 11 - SKELETON 1972
- 12 - STEWART 1974
- 13 - ST. JOHN 1974
- 14 - THREE MILE 1972
- 15 - WALKER 1974
- 16 - WASEOSA 1974

TC/100 ML



$$\text{Log}_{10} \text{TC (Spring)} = - 0.27 + 0.95 \text{ Log}_{10} \text{BKGD (Spring)}.$$

Fecal and total coliforms were correlated significantly at point inflows in the summer, but not in the spring, and not at all in the main body of the lake (Table 5). Fecal streptococcus correlated significantly with total coliforms at point inflows in both spring and summer. Since fecal coliforms are the most reliable index of fecal contamination, total coliforms cannot be used in a direct manner as a parameter of fecal pollution in lakes. However, they may be retained as a fecal indicator at streams during the summer.

#### Redundancy of Total Coliform Measurements

Total coliforms have now been correlated with summer fecal coliforms in streams, fecal streptococci, TC BKGD and Chl a in lakes. The question arose; is the measurement of total coliforms redundant? Maximum total coliform values seem to be rather well explained in terms of lake trophic status, and their use could be retained if a scale of comparison of lakes based on bacteriological measurements was required. The seasonal mean total coliform values were not fully explained by lake trophic status and the residual information may be meaningful. Total coliform bacteria should not be considered to be fully derived from growth in the lake. A more complex analysis may uncover other relationships. The correlation of total coliforms and fecal coliforms does not imply redundancy, though this has been advocated (13). Total coliform measurements could be profitably retained in surveys of rivers and point sources of pollution in lakes. It is the values from the main body of water which seem unnecessary. Those workers dealing with water samples from sewage outfalls or drinking water supplies may also feel that total coliform measurements are useful. The lake ecosystem can be described as a complex web of relationships, and so numbers of bacteria are expected to be directly and indirectly related to measurements of other organisms and substances. A parameter does not become redundant when it

merely correlates with another, but only when it ceases to perform its function, or interest in it is lost. If total coliform measurements are redundant, it is because of the lack of correlation with fecal coliforms in lakes, and so the failure of its function as a lake indicator of sewage pollution, and not as a result of the correlations with Chl a. Other uses for total coliform levels, e.g. as a measure of lake trophic status or as a means of comparing lakes, could be considered for historical data. A final decision on the redundancy of total coliform measurements cannot be made immediately.

#### The Effect of Lake Processes on The Relationship of Total Coliforms to Other Water Quality Parameters

Total coliforms were correlated with more bacterial parameters at point inflows than in the main body of the lakes (Table 5) and this indicated a change in the proportions of fecal bacteria (TC, FC and FS) after they had been washed into the lake. More correlations were maintained within lakes than across the group of lakes (Table 5 and 6) which further suggested that the factors influencing the bacteria were characteristic of the individual lakes. It is well-known that bacteria are sensitive to changes in their physical and chemical environment. The most obvious lake characteristics which would affect bacteria were temperature and nutrient levels though there are possibly others. The correlations among parameters in streams initially reflected a common source of bacteria (possibly fecal), whereas the correlations among parameters within lakes reflected the lake trophic status. The proportions of bacteria were likely modified by each lake and the changes were detected by an examination of the correlation coefficients. This important conclusion was not found in the literature though it was not really unexpected. The data supporting it were somewhat meagre and the subject will be examined as soon as possible in another set of lakes.

The bacteria from sewage pollution are omitted, usually for lack of a framework of interpretation, from studies of the effects of eutrophication of lakes on bacterial microflora. We have shown how at least one of these parameters (TC) can be influenced by nutrient levels in lakes.

#### Bacteriological Water Quality of Recreational Lakes in Muskoka

Many cottagers use their lake as a source of drinking water so it is fitting that lake water quality be evaluated with both the drinking water supply and Recreational Use Criteria, which are quoted in Table 8. Lake water quality was rated by how the total coliform values exceeded the various criteria (Table 9). Three lakes, Muskoka Bay, Maclean and Stewart, exceeded the permissible criteria for private water supplies as well as the Recreational Criteria for total coliforms. Those eutrophic lakes examined for heterotrophic bacteria exceeded the criteria for total bacteria in public surface water supplies as well (Table 2.) None of the lakes surveyed were suitable as sources of drinking water without prior disinfecting treatment.

Fecal coliform levels in the study lakes were low. The high total coliform levels, in eutrophic lakes studied, were likely due to growth of total coliforms in the lakes rather than heavy sewage pollution. These high total coliform levels, however, did indicate a measure of water quality impairment and, may emphasize a related problem, because a recent MOE study (33) showed that the ease of isolation of Pseudomonas aeruginosa, a bacterium which may cause ear and eye infections, increased in lakes of high trophic status.

#### Inhibition of Total Coliform Colony Formation by Background (BKGD) Colonies

Total coliform colony formation appears to be inhibited by certain background bacteria (BKGD). The numbers of BKGD colonies on the membrane filter can be reduced by use of less sample which leads to the appearance of

greater numbers of total coliforms. An example is illustrated using data from the 1975 survey on Lake Manitouwabing (Table 10), and the effect has been noted on many other lakes.

Total coliform levels in four lakes, Kahshe, Maclean, Waseosa, Fox, were lower in summer than in spring against the normal trend (Table 4). Background bacteria levels in these lakes was also very high (this report - Part 1), and the conditions for inhibition of total coliform colony formation may have been present. Not enough is known yet to allow a prediction of the degree to which total coliform measurements were depressed. The high BKGD levels in Bass and St. John Lakes need not have inhibited total coliform measurements as the accompanying densities of total coliforms were higher, allowing the use of less sample, with the production of less BKGD colonies on the membrane filter used for water analysis.

TABLE 8

BACTERIOLOGICAL CRITERIA FOR DRINKING WATER AND RECREATIONAL USE \*

BACTERIA	CRITERIA FOR PRIVATE WATER SUPPLIES			PUBLIC SURFACE WATER		RECREATIONAL USE
	Permissible Criteria		Desirable Criteria No Treatment	Desirable Criteria	Permissible Criteria 1)	
	Chlorination only	Chlorination and Filtration				
Total Coliforms	100/100 ml	400/100 ml	0/100 ml	100/100 ml	5,000/100 ml	1,000/100 ml
Fecal Coliforms	10/100 ml	40/100 ml	0/100 ml	10/100 ml	500/100 ml	100/100 ml
Fecal Streptococci	1/100 ml	4/100 ml	0/100 ml	1/100 ml	50/100 ml	20/100 ml
Total Bacteria	1,000/100 ml	4,000/100 ml	10/100 ml	1,000/100 ml	10 <sup>5</sup> /100 ml	-
Clostridia (in water)	0/100 ml	4/100 ml	0/100 ml	0/100 ml	50/100 ml	-

\* For conditions of sampling and other details consult ref. (16)

1) Acceptable for treatment by the defined treatment process stated in the Guideline (16).

TABLE 9

RATING OF LAKES - BY EXCEEDING DRINKING WATER AND RECREATIONAL CRITERIA FOR TOTAL COLIFORMS

NO.	LAKE	Criteria for Private Water Supplies			Public Surface Water Criteria		Recreational Criteria
		Desirable	Permissible		Desirable	Permissible	
		No Treatment	Chlorination only	Chlorination & Filtration			
1	Leonard '71	+					
2	Bala Bay '71	+					
3	Kahshe '71	+	+		+		
4	Muskoka Bay '71	+	+	+	+		+
5	Skeleton '72	+					
6	3-Mile '72	+					
7	Jerry '72 =	+					
8	Jerry '73 =	+					

+ = Criteria For Total Coliform Bacteria Exceeded For Some Portion Of The Year



Table 9 - continued

NO.	LAKE	Criteria for Private Water Supplies			Public Surface Water Criteria		Recreational Criteria
		Desirable	Permissible		Desirable	Permissible	
		No Treatment	Chlorination only	Chlorination & Filtration			
9	Harp '73	+					
10	Maclean '73	+	+	+	+		+
11	Peninsula '73	+					
12	Stewart '74	+	+	+	+		+
13	Brandy '74	+	+		+		
14	Walker '74	+					
15	Waseosa '74	+					
16	Fox '74	+					
17	Bass '74	+	+		+		
18	St. John '74	+	+		+		

+ = Criteria For Total Coliform Bacteria Exceeded For Some Portion Of The Year

TABLE 10

DILUTION EFFECT ON RECOVERY OF TOTAL COLIFORMS FROM LAKE  
MANITOUWABING, 1975.

Background Bacteria and Total Coliforms per 100 ml

Sample	Volume	1 ml	5 ml	10 ml	25 ml
Sample No.	Bacterial Type				
1	TC** BKGD	1,600 12,000		600 TNTC*	
2	TC BKGD			250 6,000	64 TNTC
3	TC BKGD			220 TNTC	64 TNTC
4	TC BKGD		1,920 5,180	270 TNTC	
5	TC BKGD		340 3,400	70 TNTC	
6	TC BKGD		100 TNTC		8 TNTC
7	TC BKGD		200 10,240	50 TNTC	
8	TC BKGD		40 TNTC	0 TNTC	

\* = TNTC - Density unknown  
too numerous to count

\*\* = TC - Total coliform colonies

BKGD = - Background colonies

ANALYSIS OF INPUTS OF TOTAL COLIFORM BACTERIA IN  
RECREATIONAL LAKES IN MUSKOKA

The main point sources of total coliform bacteria in recreational lakes were the inflowing streams. The levels of total coliforms at the mouth of streams were usually about ten times, and occasionally two hundred times, the level of total coliforms in the main body of the lake (Group A, Table 11). Other total coliform sources appeared around the shoreline. These sources were fewer in number and had lower TC levels than the sources at the inflowing streams. The geometric mean TC density for polluted streams was 422.3 TC/100 ml, while the geometric mean density for polluted locations at the shore was 157.1 TC/100 ml. The number of opportunities for an occurrence of pollution to be noted depended on the number of locations sampled and the number of surveys. This was calculated by taking the number of stations monitoring streams times the number of surveys per lake, summed for all lakes. A pollution occurrence was one in which the total coliform geometric mean density at the stream was significantly higher, as determined by an analysis of variance, than that of the main body of water into which the stream was flowing. The number of opportunities to register a pollution event was 179, and the total number of pollution events was 72. This meant that 40.2% of the stream mouths were polluted. Fecal pollution at stream mouths was measured by the more reliable fecal pollution parameter, fecal coliform. A lower number (46) of events involving fecal pollution were observed at stream mouths. Levels of fecal coliforms at streams significantly higher than those in the main body of water were observed during the surveys with a frequency of 25.7%. The data on fecal bacteria will be reported later (see Part 3).

In previous reports (e.g. 26, 27) it was noted that bacterial levels rose at stream mouths, and in the main body of the lake after rainfall. This rainfall effect could not be detected after every fall of rain, and it was speculated that a period of dry weather during which fecal material would accumulate on land was

TABLE 11

Frequency of Pollution Occurrences Measured by High Total Coliform Geometric Mean Densities at Inflowing Streams, Developed and Undeveloped Shoreline

Lake Survey	Group A GM	Number Inflow Stations	Point Sources Inflow Station		No. of Station in Developed Shoreline	No. of Station in Undeveloped Shoreline	NON POINT SOURCES (HIGH LEVELS)				NON POINT SOURCES (LOW LEVELS)			
			Station	TC/100 ml			Stations in Developed Shoreline		Stations in Undeveloped Shoreline		Stations in Developed Shoreline		Station in Undeveloped Shoreline	
							Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml
Leonard '71 spring summer fall	17.38 31.99 50.93	1 1 1			9 9 9	3 3 3			37	168.5	31	7.96	33	2.34
Bala Bay '71 spring summer fall	10.41 19.69 23.77	6 6 6			16 16 16	5 5 5	14,16 17,18 12,13	32.75 96.38	1	94.23	20	3.13		
Kashe '71 spring summer fall	37.93 10.89 325.8	4 4 4	42-44	127.4	27 27 25	9 9 9	8,9 27,29 32-34 36 40,41	148.6  547.0	35,39	547.0	22  9	15.15  99.54	15,16 46 3	43.55 226.2 162.2

TABLE 11 (continued)

- 2 -

Lake	Group A GM	Number Inflow	Point Sources Inflow Station		No. of Station in Developed	No. of Station in Undeveloped	NON POINT SOURCES (HIGH LEVELS)				NON POINT SOURCES (LOW LEVELS)			
							Stations in Developed Shoreline		Stations in Undeveloped Shoreline		Stations in Developed Shoreline		Station in Undeveloped Shoreline	
			Station	TC/100 ml			Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml
Skeleton '72 spring	2.82	11	23-25 31,32 9 12 20	53.25 74.46 101.6 41.96 78.14	18	8	37 17	25.28 36.81			38 30	1.0 1.0		
	63.36	11	43, 2 4,5 9 12 20 25 31	602.06 658.9 424.4 346.9 4816 547.0	18	5	17	359.1			38 34	10.21 15.44	40	22.26
Three Mile '72 spring	8.32	8	19,20 35 15	84.09 22.43 264.3	19	9			14	24.91	31 33 39	2.0 2.64 2.64	36	2.23
	36.71	8	8 15 20	1286 1666 778.5	19	9					34 29 23	9.61 13.15 9.49	36	5.01

94...

...45

TABLE 11 (continued)

- 3 -

Lake Survey	Group A GM	Number Inflow Stations	Point Sources Inflow Station		No. of Station in Developed Shoreline	No. of Station in Undeveloped Shoreline	NON POINT SOURCES (HIGH LEVELS)				NON POINT SOURCES (LOW LEVELS)			
			Station	TC/100 ml			Stations in Developed Shoreline		Stations in Undeveloped Shoreline		Stations in Developed Shoreline		Station in Undeveloped Shoreline	
							Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml
Jerry '72 May 21-25	44	2	11	1130	0	24								
June 16-22	39	2	12 11	121 817	0	24			21	138				
June 23-29	72	2	12 11	230 1480	0	24			21 10	330 212				
June 30- July 6	80	2	11	2930	0	24			9	196			20-24	38
July 7- 13	51	2	11	1200	0	24								
July 14- 20	68	2	11 12	2780 355	0	24			21 9	361 250				
July 23- 30	55	2	11 20-22	2110 139	0	24			9 17	118 139			25	26
Oct. 17- 21	51	2	11	198	0	24			17	142				

TABLE 11 (continued)

- 4 -

Lake Survey	Group A GM	Number Inflow Stations	Point Sources Inflow Station		No. of Station in Developed Shoreline	No. of Station in Undeveloped Shoreline	NON POINT SOURCES (HIGH LEVELS)				NON POINT SOURCES (LOW LEVELS)			
			Station	TC/100 ml			Stations in Developed Shoreline		Stations in Undeveloped Shoreline		Stations in Developed Shoreline		Station in Undeveloped Shoreline	
							Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml
Jerry '73 spring	8	2	11 12 36	145 46 118	0	24								
summer	51	2			0	24								
fall	11	2	11 36	538 615	0	24								
Harp '73 May 22- 31	26.08	4	2 7 10 13	118.7 277.2 204.6 222.5	16	1								
July 6- 12	57.57	4	2 10 13	302.8 504.0 458.1	16	1								
July 13-	75.84	4	2 10 13	469.0 732.6 368.9	16	1					18	53.19		
July 20- 26	58.34	4	7 10	522.4 876.8	16	1								

847\*\*

..47

TABLE 11 (continued)

- 5 -

Lake Survey	Group A GM	Number Inflow Stations	Point Sources Inflow Station		No. of Station in Developed Shoreline	No. of Station in Undeveloped Shoreline	NON POINT SOURCES (HIGH LEVELS)				NON POINT SOURCES (LOW LEVELS)			
			Station	TC/100 ml			Stations in Developed Shoreline		Stations in Undeveloped Shoreline		Stations in Developed Shoreline		Station in Undeveloped Shoreline	
							Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml
July 27- Aug. 2	107.1	4	2 7 10 13	1476 1029 970.7 657.5	16	1								
Aug. 3-9	53.18	4	2 13 7	522.9 650.6 515.9	16.	1	22 18,21 8,9	107.4 61.19 54.81			4	44.11		
Sept. 10- 15	24.75	4	7 10	190.1 104.0	16	1								
Maclean '73 spring	1520	4	16,17 25	167 6240	15	1								
summer	41	4	24	5820	16	1					14 19,18	1		
fall	23	4	24	4020	16	1								
Peninsula '73 spring summer	47.43 75.09	8 8			29 29	0 0								



TABLE 11 (continued)

- 6 -

Lake Survey	Group A GM TC/100-1	Number Inflow Stations	Point Sources Inflow Station		No. of Station in Developed Shoreline	No. of Station in Undeveloped Shoreline	NON POINT SOURCES (HIGH LEVELS)				NON POINT SOURCES (LOW LEVELS)			
			Station	TC/100 ml			Stations in Developed Shoreline		Stations in Undeveloped Shoreline		Stations in Developed Shoreline		Station in Undeveloped Shoreline	
							Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml
Stewart '74 spring	50.87	3	3 15 18	553.7 746.4 422.5	11	3	1 5 17	354.6 237.2 129.4						
summer	1610	3			11	3								
Brandy '74 spring	44.54	1	8	779.8	12	3								
summer	102.4	1			12	3								
Fox '74 spring	45.0	1			12	8								
summer	9.0	1	14	92.0	12	8								
Bass '74 spring	58	4	7 9	358 287	16	2								
summer	335	4	7 9 10	1400 232 1290	16	2	11 13	1290 835						

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TABLE 11 (continued)

- 7 -

Lake Survey	Group A GM	Number Inflow Stations	Point Sources Inflow Station		No. of Station in Developed Shoreline	No. of Station in Undeveloped Shoreline	NON POINT SOURCES (HIGH LEVELS)				NON POINT SOURCES (LOW LEVELS)			
			Station	TC/100 ml			Stations in Developed Shoreline		Stations in Undeveloped Shoreline		Stations in Developed Shoreline		Station in Undeveloped Shoreline	
							Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml	Station	TC/100 ml
St. John '74 spring	104.9	1	13	1559	8	7								
summer	392.7	1			8	7								
Miska- wabi '75 spring	8.92	5	14	61.28	9	4								
summer	12.92	5			9	4								
Hurri- cane '75 spring	28.11	0			5	11								
summer	16.32	0			5	11								

required before high fecal bacterial levels would follow rainfall. From the record of rainfall, recorded at MOE weather stations and not reported here, it appeared that it was not possible to predict the presence of pollution at streams based on the quantity of rainfall that had fallen before and during the survey. The stream mouths were obviously the points of inflow of most of the rural stormwater which contained from time to time much fecal material probably largely of animal origin. There was tendency for the frequency of TC pollution events to be associated with the higher levels of rainfall before surveys. It was also noted from this data that about 56% of lakes had polluted streams when surveyed (Table 12). No significant seasonal variation in the frequency of TC pollution events at stream mouths was noted (Table 13).

#### ASSOCIATION OF BACTERIAL POLLUTION WITH DEVELOPED SHORELINE IN RECREATIONAL LAKES

Shoreline pollution was considered to be of a diffuse type and was difficult to trace to a point source. Consistently high levels of bacteria were noted at shoreline locations but they were rarely linked to a shoreline source. The shoreline pollution appeared to have been spread out from its source and was termed a nonpoint source.

In order to test the association of this kind of pollution with shoreline development, the data was ordered in the following way. For this test cottages, public bathing beaches, lodges, camps, marinas, public parks or other obvious examples of lakeshore development were found on the developed shore. Undeveloped shoreline was considered free of such structures and activities. Minor signs of development, i.e. those not involving immediate human use, like road clearance, telephone poles, and culverts were ignored for this test. The sampling stations at inflows, the outfall, and midlake stations were set aside as they were considered not to be of value when examining shoreline contributions of bacteria. The

TABLE 12

Association of Pollution at Streams with Cumulative Rainfall in Lakes

Spring Data

TC Pollution at Stream Mouths				
Cumulative Rainfall before the end of Survey	Number of Lakes in which Polluted Streams were noted			Frequency % + ve
	Pollution + ve	Pollution Nil	Totals	
0" - 1.99"	1	4	5	20
2. "0 - 4.0"	11	0	11	100
TOTALS	12	4	16	75

$\chi^2 = 11.7$  . df = 1 Value significant  $P \ll 0.05$

Summer Data

TC Pollution at Stream Mouths				
Cumulative Rainfall before the end of Survey	Number of Lakes in which Polluted Streams were noted			Frequency % + ve
	Pollution + ve	Pollution Nil	Totals	
0" - 12.49"	3	9	12	25
12.5" - 18.0"	3	1	4	75
TOTALS	6	10	16	37.5
OVERALL	18	14	32	56.3

$\chi^2 = 3.2$  . df = 1 Value not significant  $P > 0.05$

TABLE 13

Seasonal Variation in Frequency of Pollution Events (TC)  
at the Mouths of Streams

Pollution (TC) at Stream Monitoring Stations

Season Lake Survey	Number of Stream Outfalls at which Pollution can be Registered		Totals	Frequency % + ve
	Pollution + ve	Pollution Nil		
Spring	29	39	68	42.6
Summer	36	52	88	40.9
Fall	7	16	23	30.4
TOTALS	72	107	179	40.2

$\chi^2 = 1.1$  . df = 2. Value not significant  $P > 0.05$

remaining stations were put into two groups, one consisting of those monitoring the developed sections of lakeshore, and the other consisting of those monitoring the undeveloped sections of shoreline. Pollution was measured by the frequency of pollution events at a sampling location, where a positive pollution event was one where the TC geometric mean density at a location along the shore was significantly higher, determined by an analysis of variance, than that of the main body of water surrounding that shoreline location. A pollution negative event was one where the TC geometric mean density was lower than the main body of water about that location. This appeared to indicate an inflow of water cleaner than that of the surrounding lake. The TC geometric mean densities at many shoreline locations were the same as the surrounding lake and they were recorded as pollution nil.

The station locations and corresponding geometric mean values are recorded in Table 11. The numbers of sampling stations with the three types of pollution events at developed and undeveloped shore in each season of the year are displayed in Table 14. These data may be simply tested by an analysis of frequency. There were some significant seasonal differences and a difference associated with shoreline development.

The frequency of polluted locations at the shoreline showed a strong seasonal variation (Table 14), unlike the frequency of such events at stream mouths (Table 13). The developed and undeveloped shore were examined separately, and a significant increase in frequency of polluted locations was noted only at the developed shore. This was the only difference between developed and undeveloped shoreline to be detected by use of the TC parameter. Pollution occurrences at stream mouths were about ten times as frequent as such events along the shore (Table 15).

The increased frequency of TC pollution in the fall of the year at the developed shore was not accompanied by increased levels of fecal coliform (see Part 3), and so could not be directly linked to fecal pollution. The link to

TABLE 14

Comparison of Seasonal Variation in Frequency of Pollution Events at Developed and Undeveloped Shore

	Season of Lake Survey	Numbers of Stations at which Pollution can be Registered				Frequency % + ve	Frequency % + ve	Range of GM Values Pollution + ve			$\chi^2$
		Pollution + ve	Pollution Nil	Pollution - ve	Totals						
Developed Shore	Spring	5	210	7	222	2.25	3.15	25	-	355	31.59 *
	Summer	10	266	11	287	3.48	3.83	55	-	1,290	
	Fall	14	67	1	82	17.07	1.21	33	-	547	
	TOTAL	29	543	19	591	4.90	3.21	-			
Undeveloped Shore	Spring	4	164	2	170	2.35	1.17	25	-	330	8.92
	Summer	5	182	6	193	2.59	3.10	118	-	361	
	Fall	5	58	4	67	7.46	5.94	94	-	547	
	TOTAL	14	404	12	430	3.25	2.79	-			

TABLE 14- continued.

	Season of Lake Survey	Numbers of Stations at which Pollution can be Registered				Frequency % + ve	Frequency % + ve	Range of GM Values Pollution + ve	$\chi^2$
		Pollution + ve	Pollution Nil	Pollution - ve	Totals				
Total Shore	Spring	9	374	9	392	2.29	2.29	25 - 355	
	Summer	15	448	17	480	3.12	3.54	55 - 1,290	
	Fall	19	125	5	149	12.75	3.35	33 - 547	33.30 *
	TOTAL	43	947	31	1021	4.21	3.03	-	df = 4

SPRING SURVEY = Any 5 consecutive days between May 1st and June 30th

\* =  $\chi^2$  significant at least  $P \leq 0.05$

SUMMER SURVEY = Any 5 consecutive days between July 1st and August 31st

FALL SURVEY = Any 5 consecutive days between September 1st and October 31st



TABLE 15

Comparison of Frequency of TC Pollution at Stream Mouths and Shoreline

Station Type	Number of Stations at which Pollution can be Registered				Frequency + ve %	Frequency - ve %	Range of GM Values Pollution + ve TC/100 ml
	Pollution + ve	Pollution Nil	Pollution - ve	TOTALS			
Streams (Point Sources)	72	107	0	179	40.2	0	42 - 4,816
Shoreline (nonpoint Sources)	43	947	31	1,021	4.21	3.03	25 - 1,290
TOTALS	115	1,054	31	1,200	9.58	2.58	-

$\chi^2 = 230.4$  . df = 2. Value significant  $P \ll 0.05$

development was likely more subtle and was perhaps due to a delayed response to nutrients added to lawns as fertilizer or originating from septic tanks. The fact that total coliforms could be shown to be influenced by lakeshore development was certainly interesting, as this was the first time that this relationship could be illustrated with this type of data.

#### FUTURE USES OF THE TOTAL COLIFORM RELATIONSHIPS WITH OTHER LAKE MEASUREMENTS

##### A) Comparison of Lakes

A method of comparing lakes based on a regression of HB on Chl a has been proposed (this report - Part 1). One disadvantage was the lack of past data, which can largely be overcome by use of total coliform values as the basis of comparing lakes. The method was used in a recent water quality report (25) in a manner similar to Figure 4 using the following regression:

$$\text{Log}_{10} \left[ \frac{\text{TC (max)}}{\text{Z (max).}} \right] = -0.67 + 1.91 \text{ Log}_{10} \text{Chl } \underline{a}$$

It is considered desirable to develop a method of comparing lakes using only bacteriological parameters, and two possible methods have been illustrated here (Fig. 5 and 6). A method utilizing all three bacteriological water quality parameters is being studied.

##### B) Lakeshore Capacity

An early objective of the Lakeshore Capacity Study was the reexamination of the traditional bacteriological water quality parameters (22). The reevaluation was initiated in this report and important conclusions were indicated which altered the interpretation of total coliform levels in lakes, and uncovered some new relationships between total coliform levels and other parameters of water quality.

Maximum survey levels of total coliforms can be estimated from mean chlorophyll levels and the data displayed in Figure 4, then linked to lakeshore development by the method previously given for heterotrophic bacteria (this report - Part 1). However, this method should be considered a method of last resort as more direct relationships will be discovered in the Lakeshore Capacity Study. The results reported here are useful for developing an understanding of the effect of lake processes on bacteria, and so contribute to an explanation of why certain bacteria are found in lakes.

The seasonal variation in the frequency of pollution by total coliforms was a feature of only the developed shoreline. This association of pollution with cottage development though slight was encouraging for further Lakeshore Capacity water quality modelling attempts.

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APPENDIX

Heterotroph Medium (Recreational Lakes)

Modified Foot and Taylor Agar

<u>A</u>	Per litre
Peptone (Difco)	3.0 g
Soluble Casein (BDH)	0.5 g
$K_2HPO_4$	0.2 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Ferric Chloride solution (0.5% aq.) solution)	0.2 ml
Agar (Difco)	20.0 g
Distilled Water	1 litre

B

Actidione - 0.5 g + 50 ml distilled water

Filter sterilize. Store at 4° C for no more than 2 days.

Preparations: Add weighed ingredients to distilled water (A) and heat to boiling with agitation to dissolve. Adjust pH to 7.05. Autoclave 15 minutes 15 psi. Then cool to 50°-55°C and add 10 ml Actidione solution (B) to give 100 ppm Actidione. Mix well, and dispense in sterile plates. Final pH will be 7.2.

Note This medium is very similar to that used by the Federal Dept. of the Environment. (See Methods for Microbiological Analysis of Water, Wastewater and Sediments, CCIW, 1976, by B. J. Dutka.



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